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PATENT

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January 15, 2002 rap — Date

Rozell A. Price rap — Rozell A. Price

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	William Butler Cowden, Kevin John Lafferty Lawrence Scott Gazda	**Examiner:**	S. Devi, PhD.
Serial No.:	09/142,597	**Art Unit:**	1641
Filed:	September 10, 1998	**Docket:**	120081.403
For:	USE OF COXIELLA BACTERIA TO TREAT AUTOIMMUNE DISEASE	**Date:**	January 8, 2002

Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C., 20231

DECLARATION OF WILLIAM BUTER COWDEN UNDER 37 CFR §1.132

Sir

I, William Butler Cowden, hereby declare as follows:-

1. I am a co-inventor of the subject matter described and claimed in the above-identified application.

2. I have reviewed the Office Action dated March 21, 2001, in the subject application, including the rejections under 35 USC §112, first paragraph, and 35 USC §103(a), and provide this Declaration for the purpose of assisting the Examiner in evaluating the teachings of the specification and to confirm data in support of various fractions of *Coxiella burnetii* being effective in the treatment of insulin-dependent diabetes mellitus (IDDM).

3. The experiments presented herein were performed by one or more of the inventors, by those under my direct supervision, or are within my personal knowledge.

4. In the experiments set forth herein, the ability of specific fractions of *Coxiella burnetii* to treat IDDM is demonstrated. First, there is clear evidence that the residue (CMR) remaining after delipidation of whole *C. burnetii* is very effective, in a highly statistically significant manner at preventing the onset or development of IDDM in the NOD mouse (see Figure 1 ***attached***), while the components of the extracted lipidic material (CME) are without effect. Analysis of the CMR fraction showed it to be 26% protein and 9% carbohydrate by weight. The CME fraction, as expected was largely lipid and consisted of only about 1% protein and 3% carbohydrate by weight. The preparation of these fractions has been described in the literature (Williams *et al., Infection and Immunity 51:* 851-858, 1986).

5. A DMSO extract of the whole cell *C. burnetii* was prepared as follows. The contents of 5 vials of QFA (CSL, Australia) was centrifuged at 13,000 rpm/SS34/4'C/20min (ca. 16000 x g). The resulting pellet (**P1**) was extracted with DMSO (20ml) at ca.55°C for 24h. The resulting suspension was centrifuged at 20 x g for 20 min. The clear supernatant (DMSO extract) was collected and dialyzed against water (2L x 2). The pellet (DMSO residue) was washed with DMSO (5 ml) by vortex mixing, followed by centrifugation at 20 x g. The supernatant (DMSO extract) was collected and combined with the first extract and lyophilized. The DMSO residue was suspended in water and dialysed against water (2L x 2). The residue was then lyophilized. The extract fraction consists of 20% protein and 27% carbohydrate by weight and a substantial amount of lipid material. Both fractions were examined for major protein components, thus, silver stained SDS-PAGE gels (reducing) revealed a major band at ca. 30 kD, 5 bands between 6.5 to 21 kD and a band at the dye front in the extract. The DMSO residue produced several bands having similar intensities. The band at 30kD is not a major band in any of the other antigen preparations.

6. The DMSO extract was effective in preventing the onset of IDDM in NOD mice. Thus, at 183 days old, none of the DMSO extract (105 mcg, ip as per Examples 1 and 2 of the above-identified application) treated mice had become diabetic. Thus the DMSO extract fraction prevented the onset of diabetes by 100%. Specifically, 0% of treated animals (0/5) at 180 days had developed diabetes versus 60% (3/5) of the saline treated control mice at 180 days of age.

7. The LPS fraction of *C. burnetii* (prepared as described by Schramek and Galanos, *Wcta Virol. 25:* 230-234, 1981) was also isolated and tested for its ability to inhibit the development of IDDM in the NOD mouse and found not to be active.

8. In summary, following the teachings of the specification, we readily conclude that a variety of fractions of *Coxiella burnetii* may prevent, inhibit, delay onset of or ameliorate the effects of a particular autoimmune disease.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9 JANUARY 2002
Date

William Butler Cowden
56 Urambi Village
Kambah, Australian Capital Territory, 2902
Australia

Enclosures: Postcard
Figure 1

Seed Intellectual Property Law Group PLLC
701 Fifth Avenue
Suite 6300
Seattle, Washington, 98104-7092
Tel: (206) 622 4900
Fax: (206) 682 6031

Figure 1. Incidence of diabetes in NOD mice treated with CMR or CME